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Differences in Susceptibility of Inbred Mice to *Bacillus anthracis*

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Animal species differ in their resistance both to infection by *Bacillus anthracis* and to anthrax toxin. A mouse model was developed to study the basis of the host differences and the pathogenesis of infection. When mice were infected with the virulent *B. anthracis* strain Vollum 1B, low 50% lethal dose (LD_{50}) values (5 to 30 spores) were found for all 10 strains of inbred mice tested. However, analysis of time-to-death data revealed significant differences among the strains, which could be divided into three groups: most susceptible (A/J and DBA/2J); least susceptible (CBA/J, BALB/cJ, and C57BR/cdJ); and intermediate (the remaining five strains). In contrast, the mice were distinctly susceptible or resistant to lethal infection by the toxigenic, nonencapsulated Sterne vaccine strain. The LD_{50} for the susceptible A/J and DBA/2J mice was approximately 10^3 spores of the Sterne strain, whereas the remaining eight relatively resistant strains were killed only by 10^6 or more spores. F_1 hybrid and backcross studies suggested that resistance to the Sterne strain is determined by a single dominant gene or gene complex. Mice lethally infected with *B. anthracis* showed an acute course of infection, characterized by extensive gelatinous edema and large concentrations of bacilli in the blood and organs (e.g., 10^9 CFU/g of spleen). The susceptibility of A/J and CBA/J mice to intravenously injected anthrax toxin components appeared to differ from their susceptibility to infection. The toxin LD_{50} values for both strains were similar. However, CBA/J mice died sooner than did A/J mice, with mean time to death of 0.9 and 3.7 days, respectively, in mice given 4 LD_{50} of toxin. The mouse model appears to be useful in studies on host resistance to anthrax and on the pathogenesis of the infection.

Bacillus anthracis, the agent of anthrax, causes disease primarily in domestic and wild animals. However, it can produce either cutaneous anthrax or an often fatal systemic disease (inhalation or gastrointestinal anthrax) in people exposed to infected animals or their products (4, 6, 15, 23). The two major virulence factors of *B. anthracis* are a poly-D-glutamic acid capsule and an exotoxin composed of the three protein components protective antigen (PA), lethal factor (LF), and edema factor (3, 8, 9, 18, 23, 36, 37, 39).

The pathogenesis of infection by *B. anthracis*, the role of toxin in the disease, and the mechanisms of host defense are poorly understood. Previous studies indicated that animal species vary in their susceptibility to lethal infection with the spores and in their susceptibility to killing by intravenous (i.v.) doses of the toxin (9, 14, 17, 19, 21, 25, 40). Outbred mice and guinea pigs succumb to low parenteral doses of *B. anthracis* (50% lethal dose [LD_{50}] values of 5 and 50 spores, respectively), whereas the corresponding LD_{50} for rats is approximately 10^6 spores (19, 21, 25, 40; J. Jemski, unpublished data). Anthrax toxin can be detected in the blood late during a lethal infection, and its concentration increases in parallel with that of the toxin-producing organisms. The terminal concentration of toxin in the blood of infected animals correlates with the resistance of that species to i.v. doses of toxin. Animals such as the rat, which are relatively resistant to lethal infection, appear to be more susceptible to killing by injected toxin than are those that succumb to a small infectious dose. For example, immediately prior to death, infected guinea pigs had 50 U of toxin (and 10^8 CFU) per ml of blood, whereas the blood of moribund rats yielded less than 8 U of toxin (and only 10^4 CFU) per ml (23, 25). Lincoln et al. (25) hypothesized that there is an inverse relationship between the ability of a host to resist the establishment of *B. anthracis* and the ability to resist the lethal effects of the toxin produced subsequently by the organisms; i.e., animals resistant to infection are susceptible to toxin, and vice versa. It is also possible that the mechanisms responsible for resistance to infection and to intoxication are unrelated and function independently. Regardless, the data suggest that (i) resistance to infection and to the lethal effects of toxin might involve separate mechanisms (21, 25), and (ii) animals resistant to infection have mechanisms which interfere with initial germination or multiplication, or both, preventing systemic accumulation of the lethal toxin.

To study questions of pathogenesis and host resistance, a model which stimulates the range of responses of different animals to anthrax is needed. Abalakin et al. provided some evidence for differences in resistance among inbred mice (1). Several strains were killed by 400 spores of a fully virulent encapsulated strain of *B. anthracis*. However, mouse strain CC57BR survived challenge with a 100-fold-higher dose of spores. When challenged with a nonencapsulated, toxin-producing vaccine strain (ST1), two mouse strains, A/Sn and DBA/2, died, whereas the other mouse lines tested were resistant. The purpose of our work was to determine the suitability of inbred mice as a model for studying infection by *B. anthracis*. We characterized individual strains of mice that differ significantly in their susceptibility to infection by *B. anthracis* and to lethal intoxication.

MATERIALS AND METHODS

Mice. C3H/HeN mice were purchased from Harlan Industries, Inc., Indianapolis, Ind. A/J, DBA/2J, CBA/J, C57L/J, C58/J, C57BL/6J, C3H/HeJ, BALB/cJ, and C57BR/cdJ mice were purchased from Jackson Laboratories, Bar Harbor, Maine. The F_1 hybrid and backcross mice used were bred by Jackson Laboratories. Female mice that were

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6 weeks old and that weighed approximately 18 to 22 g each were used in all experiments, except where indicated.

(In conducting the research described in this report, we adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.)

Bacterial strains and media. A toxigenic encapsulated strain (Vollum 1B), a toxigenic nonencapsulated strain (Sterne), and a nontoxigenic encapsulated strain (Pasteur 6602) of *B. anthracis* were obtained from the culture collection of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Md. Strain VNR-1, obtained from B. Ivins, USAMRIID, is a rough derivative of strain Vollum 1B that was cured of a plasmid required for capsule production by growth in the presence of novobiocin (12). Spore preparations of each strain were made by using the broth medium and growth conditions described by Leighton and Doi (22). Cultures containing at least 90% spores and 10^9 CFU/ml were collected by centrifugation at 4,000 × *g* for 20 min, washed (twice) in sterile water, and suspended in a volume of water equal to approximately 1/50 the original culture volume. Dilutions of the spore stock were plated for viable counts on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) plates, before and after heating the stock at 68°C for 30 min to destroy vegetative bacilli. Plates were incubated at 37°C in an atmosphere of 5% CO_2 in air. Glycerol was added to 10% (vol/vol), and the spore stock was frozen in aliquots at −70°C. Prior to each infection experiment, a frozen aliquot was thawed and diluted in Hanks balanced salt solution, and the dilutions were spread on Trypticase soy agar plates for viable count determinations.

Virulence testing of spores. Mice were inoculated with 0.2-ml volumes of spores. Except where otherwise noted, mice were inoculated subcutaneously. Ten mice were infected with each dose of organisms. They were observed for 14 days, and the LD_{50} was calculated by Probit analysis with the Computerized Biostatistical Analysis Library, USAMRIID, or by the graphic method of Litchfield and Wilcoxon (26). The time to death (TTD) for each mouse was also recorded, and the geometric and harmonic mean TTD were calculated for each dose of *B. anthracis* (10, 31). The harmonic mean TTD is determined by the following: $TTD = N/\Sigma(1/TTD)$; where N is the total number of animals infected per dose and TTD is given in days, with TTD equal to infinity for survivors. Two methods were used to perform regression analysis of the dose-dependent TTD for each mouse strain. In the first, reciprocal harmonic mean TTD values were determined for each dose and strain and then compared by using computer programs (Statistical Analysis System, SAS Institute, Cary, N.C., 1982). For the second analysis, Cox's proportional hazards model was used to estimate the probability of survival of the infected mice with time after inoculation (SAS program BMDP2L, BMDP Statistical Software, University of California, Berkeley, Calif., 1983). The Cox method models the survival instead of TTD of infected mice and is based on instantaneous rates of death ("hazard" rates) relative to the given covariates of mouse strain and dose of infecting organism. Mouse susceptibility groups were determined in both regression analyses by multiple pairwise comparisons by the Bonferroni method (27). The susceptibility rankings of the mouse strains derived from both methods were the same.

Virulence testing of toxin components. Purified LF and PA components of anthrax toxin and affinity-purified goat anti-PA antibody were gifts of S. Leppla, USAMRIID. PA and LF were combined in a ratio of 5:1 (wt/wt), and serial twofold dilutions of the mixture were prepared and injected into A/J and CBA/J mice via the tail vein, as described by Ezzell et al. (9). Five mice per strain were inoculated with each dilution of the mixture or with PA, LF, or diluent alone; the experiments were done twice. Heart blood collected from necropsied mice was spread on sheep blood agar medium, and the plates were incubated at 37°C in air with 5% CO_2. The TTD values were recorded, and the LD_{50} was determined as given above for the infection experiments. The TTDs of A/J compared with CBA/J mice were analyzed statistically with Computerized Biostatistical Analysis Library programs for analysis of variance and Fisher's least-significant-difference test.

The biological activity of the PA and LF preparation used was confirmed by the sensitive assay of anthrax toxin lethality for rats (3, 9). Rats injected i.v. with doses containing 12 μg of PA and 2.4 μg of LF (ca. 4 LD_{50}s) died within 106 min. When these doses were preincubated with 120 μg of affinity-purified goat anti-PA antibody, the rats were protected from lethal toxicity.

Necropsy and specimen collection. Mice were killed by intramuscular injection of 5 mg of ketamine hydrochloride (Vetalar; Parke-Davis, Morris Plains, N.Y.) and 1 mg of xylazine (Rompun; Miles Laboratories, Shawnee, Kans.) in 50 μl and were dissected immediately. Gross pathological changes were noted, heart blood and subcutaneous edema fluid were collected by needle aspiration, and organs were removed and weighed for quantitative culture or processed for staining with hematoxylin and eosin. Edema fluid and blood specimens were smeared on slides for staining with Giemsa and Gram stains. Specimens to be cultured were homogenized and diluted in 0.4% Na_2HPO_4 (pH 7.0) with 0.2% gelatin (17) and spread on Trypticase soy agar plates.

RESULTS

Ten strains of inbred mice were screened for differences in susceptibility to lethal infection by *B. anthracis*. Table 1 shows the response of mice to inoculation with the fully virulent encapsulated and toxin-producing Vollum 1B strain of *B. anthracis*. Following subcutaneous or intraperitoneal challenge with the virulent organism, all the mice were killed

TABLE 1. Susceptibility of mice to virulent *B. anthracis* Vollum 1B

Mouse strain	LD_{50} (no. of spores)[a]	
	s.c.	i.p.
A/J	5.5 (2.6)[b]	41
DBA/2J	<6	
C3H/HeN	<6	
C3H/HeJ	5.6	
BALB/cJ	6.6	
C58/J	9	
C57BL/6J	14.5	
C57L/J	22	
CBA/J	25 (3.4)	151
C57BR/cdJ	30 (1.3)	

[a] LD_{50} values obtained by subcutaneous (s.c.) and intraperitoneal (i.p.) routes of inoculation are shown.

[b] LD_{50} values followed by values in parentheses indicate geometric mean (geometric standard deviation) of two to three experiments.

by relatively low doses: the subcutaneous doses (LD_{50}) ranged from 5 to 30 spores.

Despite the similarity in the lethal doses of Vollum 1B for the different mouse strains, the TTD of the mice differed significantly. A pattern of incremental or continuous variation in TTD was observed (Table 2) (32, 35). Strains CBA/J, BALB/cJ, and C57BR/cdJ (group 3) were the most resistant; the mice survived for 5.4 to 6.5 days after subcutaneous inoculation with the mean dose (60 spores). The A/J and DBA/2J strains (group 1) were the least resistant. The mice died within about 3 days after infection. The mice in group 2 had TTD values intermediate between those of the two significantly different groups. The probability of survival as a function of time was analyzed for each strain by using the Cox statistical model. The data (not shown) confirmed the significant differences observed in TTD.

The pathologic and bacteriologic findings in mice lethally infected with Vollum 1B were similar to those previously observed in anthrax-infected animals (15, 17, 19, 21, 23, 24, 40). Prior to death, the mice became wasted and developed paralysis and often extreme swelling near the site of inoculation. Typical findings on autopsy included splenomegaly, a gelatinous and often hemorrhagic subcutaneous exudate, and dark, thickened blood. Histologic findings were as previously described (19, 23, 24, 40). Stained heart blood specimens revealed distorted erythrocytes and numerous encapsulated gram-positive bacilli. The latter were usually present as single or duplex rods; longer chains of bacilli were often observed in the edema fluid specimens. Bacteremia was confirmed by culture, and all organs sampled from the mice (spleen, liver, lungs, and blood) were positive for *B. anthracis* during the infection period of rapidly increasing mortality. High concentrations of bacteria were observed, particularly in the spleen, where bacilli often exceeded 10^9 CFU/g.

The mice were also screened for their susceptibility to nonencapsulated toxin-producing strains of *B. anthracis*. Infection of mice with lethal doses of the Sterne vaccine strain caused pathologic and bacteriologic signs in the final stages of disease that were similar to those observed with Vollum 1B. In contrast to their susceptibility pattern for Vollum 1B, the mice were either susceptible or relatively resistant to lethal infection with these organisms. There was a 10^3- to 10^4-fold difference between the LD_{50}s for two susceptible strains (A/J and DBA/2J) and the LD_{50}s for the remaining eight relatively resistant strains (Table 3). VNR-1, a toxigenic nonencapsulated derivative of Vollum 1B, demonstrated similar lethality for mice. TTD values were also determined for mice infected with Sterne, and they corroborated the division of mouse strains by LD_{50} into susceptible groups (group 1, A/J; group 2, DBA/2J) and a resistant group (group 3, comprising the remaining strains) (Fig. 1). In addition, these data indicate that the A/J and DBA/J mice are not equally susceptible, in that A/J mice succumbed to infection more rapidly than did DBA/2J mice (Fig. 1).

The clear division between relatively resistant mice and susceptible mice indicates that resistance to the Sterne strain may be under the control of one major gene. To investigate a genetic linkage, F_1 mice were obtained from matings between A/J and each of three resistant strains (C57BL/6J, CBA/J, and BALB/cJ). The hybrid mice were infected with spores of the Sterne strain at a dose which kills the majority of A/J mice but not the more resistant mice. The response of the F_1 progeny from each mating was indistinguishable statistically from that of the resistant parent strain. No sex-linked or sex-influenced component was detected in the response of F_1 mice from reciprocal matings of A/J and CBA/J and of male and female F_1 progeny from one mating. Next, the (A/J × CBA/J)F_1 hybrids were crossed to the parental susceptible (A/J) and resistant (CBA/J) strains. The progeny resulting from the backcross to CBA/J mice were uniformly resistant to Sterne, whereas nearly half (47.6%) of the mice from the backcross to A/J succumbed to Sterne infection (Table 4). The mortality rates for male and female backcross mice were not significantly different. Based on these results, host resistance to Sterne appears to be dominant and associated with a single autosomal gene or gene complex.

The susceptibility of the mouse strains to anthrax toxin was studied to verify a role for toxin in this mouse model and to determine the relative susceptibilities of the strains to toxin. Strains A/J and CBA/J were injected i.v. with preparations of PA antigen and LF mixed in a 5:1 (wt/wt) ratio, as described by Ezzell et al. (9). The toxin LD_{50} for the two

TABLE 2. Differences in susceptibility of mice to lethal infection with virulent *B. anthracis* Vollum 1B

Susceptibility group[a]	Mouse strain	TTD (days)[b]
1	DBA/2J	3.0 (2.6–3.7)
	A/J	3.2 (2.8–3.6)
2	C3H/HeN	3.5 (2.8–4.8)
	C57BL/6J	4.1 (3.2–6.0)
	C3HHeJ	4.2 (3.3–5.7)
	C57L/J	4.4 (3.3–6.5)
	C58/J	5.2 (3.7–8.3)
3	CBA/J	5.4 (4.5–6.8)
	BALB/cJ	5.5 (4.1–6.7)
	C57BR/cdJ	6.5 (5.0–9.3)

[a] Mice were divided into three significantly different groups ($P = 0.002$) by multiple pairwise comparisons (Bonferroni method) of the regression coefficients for each mouse strain (data not shown). Comparisons were made at the geometric mean subcutaneous dose (60 spores) of Vollum 1B and were made relative to C57BL/6J. Analysis of covariance had confirmed that the slopes of the regression curves for each strain were not statistically different.

[b] Harmonic mean TTD of mice infected subcutaneous with the geometric mean dose of spores grown in Leighton Doi broth. Values in parentheses correspond to the 95% confidence interval over the TTD.

TABLE 3. Susceptibility of mice to nonencapsulated strains of *B. anthracis*

Mouse strain	Susceptibility group[a]	LD_{50} (no. of spores) of challenge strain	
		Sterne	VNR-1
A/J	S	1.1×10^3 (1.1)[b]	1.6×10^3
DBA/2J	S	2.0×10^3	
C57BL/6J	R	8.6×10^5 (2.8)	
C3H/HeN	R	8.3×10^6	
CBA/J	R	2.1×10^7 (4.7)	1.8×10^7
BALB/cJ	R	6.8×10^7	
C58/J	R	$>4 \times 10^7$[c]	
C3H/HeJ	R	$>2 \times 10^7$[c]	
C57BR/cdJ	R	$<1 \times 10^8$[d]	
C57L/J	R	$<1 \times 10^8$[d]	

[a] Relatively susceptible (S) or relatively resistant (R) to killing after subcutaneous inoculation with *B. anthracis* Sterne or VNR-1.

[b] For explanation, see footnote *a*, Table 1.

[c] Highest dose yielding 100% survival. LD_{50} not determined.

[d] Mice were challenged with two doses of spores. All mice survived doses of 1×10^5 to 2×10^5 spores, and all were killed by 1×10^8 to 2×10^8 spores.

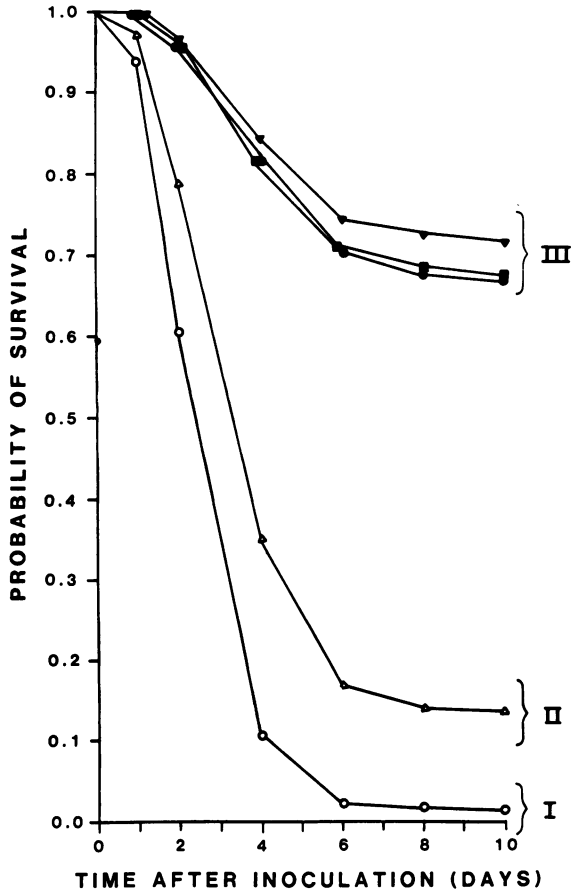

FIG. 1. Probability of survival of mice infected with the geometric mean dose (5×10^5 spores) of the Sterne strain as a function of time. The probability is shown from 1.0 (100% survival) to 0 (0% survival). Cox's proportional hazards method was used to model survival of the mice, as described in Materials and Methods. The mouse strains were divided into three significantly different groups ($P = 0.0005$): two susceptible groups (group 1 and 2; I and II in figure) and one resistant group (group 3; III in figure). Symbols: ○, A/J; △, DBA/2J; ▼, C57BL/6J; ■, CBA/J; and ●, C3H/HeN.

strains were similar: 11.0 μg of PA/2.2 μg of LF for A/J and 12.4 μg of PA/2.5 μg of LF for CBA/J mice. However, the CBA/J animals died more rapidly than did the A/J mice, as shown in Fig. 2, which depicts the cumulative mortality in toxin-treated groups having 80 to 100% mortality. The mean TTD of A/J and CBA/J mice given 8 LD_{50}s, 100 μg of PA/20 μg of LF, were 3.1 and 1.1 days, respectively; corresponding values for mice given 4 LD_{50}s were 3.7 and 0.9 days, respectively. Mice injected with PA, LF, or diluent alone survived. Heart blood collected from four mice dying between 6 h and 5 days after injection yielded no growth on blood agar.

In vivo production of toxin by *B. anthracis* presumably has a major role in the death of the mice, as it does for other animals (23, 37). Encapsulated but nontoxigenic *B. anthracis* spores were avirulent for our animals. Thus, all of the A/J mice inoculated with doses up to 10^6 spores (highest dose tested) of the Pasteur 6602 strain (capsule +, toxin −) survived the infection (data not shown).

DISCUSSION

Systemic anthrax is a rapidly progressive and lethal disease caused by toxigenic and encapsulated strains of *B. anthracis* (4, 6, 15, 23). Animal species differ considerably in their natural resistance to this disease, and empirical data have suggested that herbivores are especially susceptible. Studies with laboratory animals have confirmed the large variation in host resistance (14, 17, 21, 25, 40). However, the mechanisms of both host resistance to and pathogenesis of the disease remain obscure.

Development of a lethal infection by *B. anthracis* requires germination of the spore inoculum, systemic invasion, multiplication, and toxin production leading to death. Host resistance mechanisms could conceivably interfere with any of these steps. For instance, Hachisuka showed that spores of *B. anthracis* injected intraperitoneally into outbred mice germinate rapidly and multiply, whereas the spores are phagocytosed and destroyed in the rat peritoneum (14). It was suggested that nutrients that stimulate germination are present in the former but not the latter site of inoculation (14, 17, 21, 34). The factors determining in vivo outgrowth versus

TABLE 4. Backcross analysis of susceptibility of mice to *B. anthracis* Sterne

Mice[a]	Total no.	No. (%) observed dead[b]	χ^2	P_c
$F_1 \times$ A/J				
Females	29	15 (51.7)	0.115	NS
Males	34	15 (44.1)		
$F_1 \times$ CBA/J				
Females	30	0 (0)	0	NS
Males	29	0 (0)		

[a] F_1 mice are the (A/J × CBA/J)F_1 progeny, and the dose of Sterne given was 3×10^5 spores.

[b] The total percentage of mice that died was 47.6 and 0 for $F_1 \times$ A/J mice and $F_1 \times$ CBA/J mice, respectively. The total number and percentage of dead mice expected for a trait controlled by a single dominant gene is 31.5 (50%) and 0 (0%) for the $F_1 \times$ A/J and $F_1 \times$ CBA/J mice, respectively.

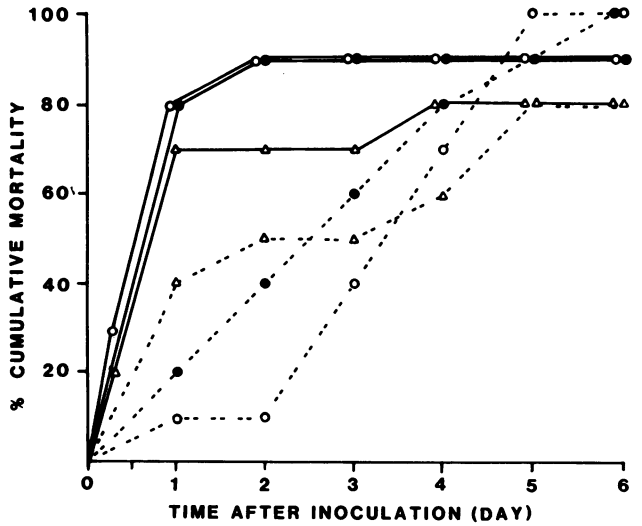

FIG. 2. Cumulative mortality of mice injected i.v. with doses of PA and LF. CBA/J mice (——) and A/J mice (– – –) were inoculated with the following mixtures, given in micrograms of PA per microgram of LF: 100/20 (●), 50/10 (○), or 25/5 (△). The mean TTD of A/J compared with CBA/J for the two highest doses of PA/LF were significantly different at $P < 0.01$ and $P < 0.001$, respectively.

phagocytic destruction of spores are unclear, as are the host responses during the stages of systemic invasion and terminal toxemia (14, 24, 33, 40).

In the present study, we examined inbred mice as a model for studying anthrax, specifically the variation in host susceptibility to infection by *B. anthracis*. The course of disease and pathology in lethally infected mice resembled that in other animals with anthrax (15, 17, 19, 21, 23, 24, 40), and the susceptibilities of 10 mouse strains to infection were characterized. In contrast to previous findings by Abalakin et al. (1), none of the mouse strains examined was resistant to lethal infection with capsule- and toxin-producing *B. anthracis* (Vollum 1B). Nevertheless, group 3 mice (Table 2) had clearly prolonged survival times in comparison with the more rapidly killed group of mice (group 1). The intermediate responses of the group 2 mice suggest that susceptibility to Vollum 1B varies in a continuous manner and that the host response to virulent *B. anthracis* is complex. Strains of susceptible and relatively resistant mice are being used for investigations on, for example, host responses to spore challenge and the physiological conditions required for germination, the lethal activity of anthrax toxin, and the protective effects of improved vaccines.

In contrast to the results with the virulent organism, the mice were either susceptible or resistant to the attenuated strains. Most animals have a mechanism to control infection by Sterne and related strains of *B. anthracis* that are used as vaccines (2, 15, 38). This function appears to be defective in A/J and DBA/2J mice and suggests that resistance to the nonencapsulated, toxigenic strains might be under the genetic control of one or a few genes (32, 35). F_1 mice were obtained from matings between A/J and several resistant strains of mice. The susceptibilities to *B. anthracis* Sterne of the F_1 mice and of mice from the backcross of (A/J × CBA/J)F_1 to each parent suggest that resistance to Sterne is genetically linked to one major dominant autosomal locus or gene complex.

The strains of mice susceptible to the Sterne strain (A/J and DBA/2J) have certain abnormalities in host defense mechanisms, yet do not exhibit a uniformly increased susceptibility to infectious agents (5, 30, 32) or to anthrax toxin (see below). For instance, the mononuclear phagocytes of A/J mice are defective in several functional parameters (5); however, these mice are resistant to certain organisms for which the macrophage plays a major role (13, 16, 28). In contrast, A/J mice are susceptible to *Listeria monocytogenes*. Susceptibility to *L. monocytogenes* in mice has been genetically linked to innately low levels of peritoneal macrophages, to poor macrophage inflammatory responses, and to hereditary deficiency of the C5 component of complement, encoded by the *Hc* gene (11). An association between C5 levels and impaired host defense to *Staphylococcus aureus* in DBA/2J mice has also been observed (7). Both A/J and DBA/2J mice are deficient in C5 and have defective *Hc* genes. *In vitro* data have suggested a role for complement in resistance to *B. anthracis*. O'Brien et al. (29) showed that normal rabbit serum, but not heat-treated serum, can opsonize the Sterne strain and allow phagocytosis by human polymorphonuclear neutrophils. Congenic or recombinant inbred mice derived from resistant and susceptible strains will be needed to establish linkage between the susceptibility to Sterne and a specific gene or host function.

Identification of the genetic basis of susceptibility to nonencapsulated *B. anthracis* in mice would be of practical value in vaccine studies. The efficacy of live, attenuated strains in immunization of animals suggests that they might be superior to the PA vaccine used in man (15, 20, 38). The latter induces antibody levels that are low and transient (15, 23). Inbred mice could be used to screen new live vaccine strains for one which protects mice, such as the A/J animals, against challenge with virulent organisms without itself causing disease.

The toxigenic nature of anthrax was demonstrated more than 30 years ago (36). However, the molecular action of anthrax toxin in vivo is still unknown (15, 23, 37). As discussed above, past studies showed that animal species which are relatively resistant to lethal infection are killed by much smaller doses of toxin than are species which are susceptible to infection (9, 21, 23, 25, 37). These different responses to toxin and to infection in an animal suggest that separate mechanisms are involved in host defense against spore challenge and lethal toxicity. We studied the susceptibility of mice to anthrax toxin, the A/J and CBA/J strains representing ones that are relatively susceptible or resistant, respectively, to infection. The toxin LD_{50} values for these mice were similar and agreed with the lethal dose obtained in outbred mice: 12.5 μg of PA combined with 2.5 μg of LF (9). However, CBA/J mice were killed more rapidly by toxin than were the more spore-susceptible A/J animals. A macrophage cytotoxicity test for LF has been developed, and it demonstrated that macrophages derived from CBA/J mice are killed at lower doses of toxin (PA + LF) than those from A/J mice (A. M. Friedlander, personal communication). These findings are therefore similar to the inverse relationship between susceptibility to toxin and to infection found in outbred animals.

In summary, the inbred mouse strains examined differed significantly in their susceptibility to lethal infection by *B. anthracis* and to lethal toxicity. The graded response of the strains to an encapsulated, toxigenic *B. anthracis* strain implies that the host response to anthrax is multifactorial. However, the mice were distinctly resistant or susceptible to the nonencapsulated bacilli, and genetic studies suggested that resistance to these organisms is controlled by a single dominant locus or gene complex. The susceptibility of two strains to toxin challenge was the inverse of that to infection and requires further study. This mouse model provides a system to investigate the pathogenesis and mechanisms of host resistance to anthrax.

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